Designation: E2911 – 23

Standard Guide for
Relative Intensity Correction of Raman Spectrometers[1]

This standard is issued under the fixed designation E2911; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide is designed to enable the user to correct a Raman spectrometer for its relative spectral-intensity response function using NIST Standard Reference Materials[2] in the 224X series (currently SRMs 2241, 2242, 2243, 2244, 2245, 2246), or a calibrated irradiance source. This relative intensity correction procedure will enable the intercomparison of Raman spectra acquired from differing instruments, excitation wavelengths, and laboratories.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 Because of the significant dangers associated with the use of lasers, ANSI Z136.1 or suitable regional standards should be followed in conjunction with this practice.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]
E131 Terminology Relating to Molecular Spectroscopy
E1840 Guide for Raman Shift Standards for Spectrometer Calibration

E2529 Guide for Testing the Resolution of a Raman Spectrometer
2.2 *ANSI Standard:*[4]
Z136.1 Safe Use of Lasers

3. Terminology

3.1 *Definitions*—Terminology used in this practice conforms to the definitions in Terminology E131.

4. Significance and Use

4.1 Generally, Raman spectra measured using grating-based dispersive or Fourier transform Raman spectrometers have not been corrected for the instrumental response (spectral responsivity of the detection system). Raman spectra obtained with different instruments may show significant variations in the measured relative peak intensities of a sample compound. This is mainly as a result of differences in their wavelength-dependent optical transmission and detector efficiencies. These variations can be particularly large when widely different laser excitation wavelengths are used, but can occur when the same laser excitation is used and spectra of the same compound are compared between instruments. This is illustrated in Fig. 1, which shows the uncorrected luminescence spectrum of SRM 2241, acquired upon four different commercially available Raman spectrometers operating with 785 nm laser excitation. Instrumental response variations can also occur on the same instrument after a component change or service work has been performed. Each spectrometer, due to its unique combination of filters, grating, collection optics and detector response, has a very unique spectral response. The spectrometer dependent spectral response will of course also affect the shape of Raman spectra acquired upon these systems. The shape of this response is not to be construed as either "good or bad" but is the result of design considerations by the spectrometer manufacturer. For instance, as shown in Fig. 1, spectral coverage can vary considerably between spectrometer systems. This is typically a deliberate tradeoff in spectrometer design, where spectral coverage is sacrificed for enhanced spectral resolution.

4.2 Variations in spectral peak intensities can be mostly corrected through calibration of the Raman intensity (y) axis.
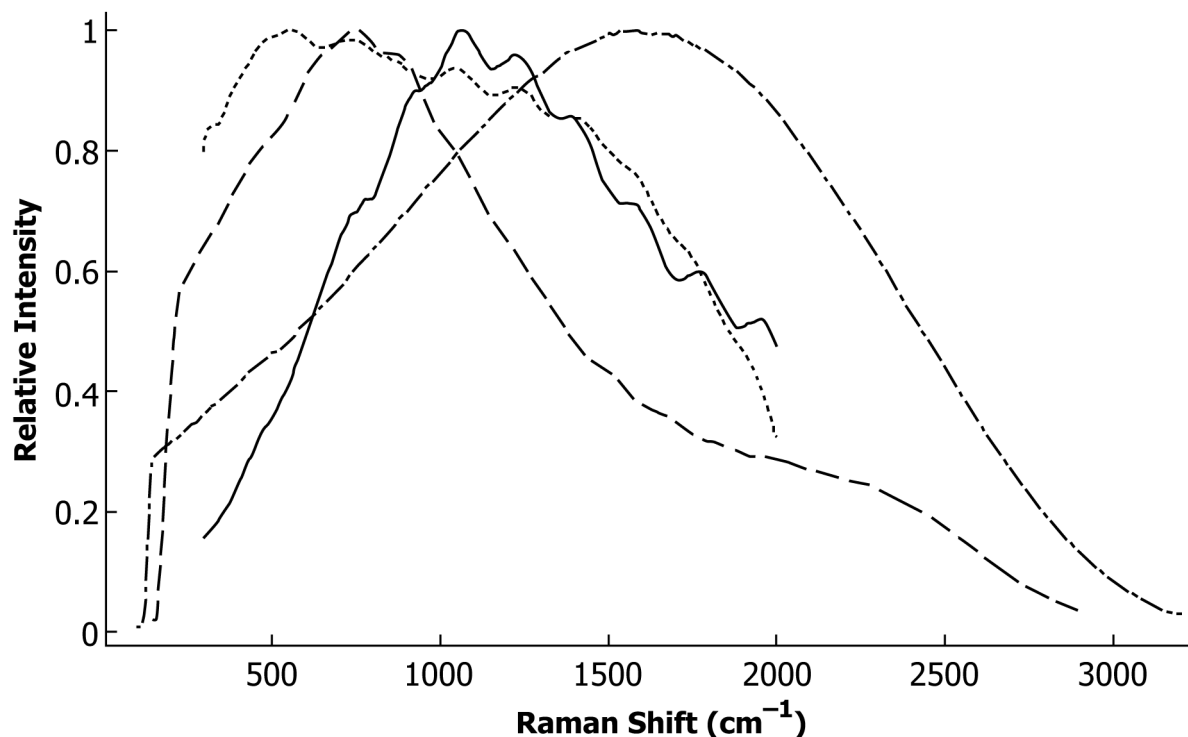

FIG. 1 SRM 2241 Measured on Four Commercial Raman Spectrometers Utilizing 785 nm Excitation

The conventional method of calibration of the spectral response of a Raman spectrometer is through the use of a National Metrology Institute (NMI), for example, NIST, traceable calibrated irradiance source. Such lamps have a defined spectral output of intensity versus wavelength and procedures for their use have been published (**1**)[5]. However, intensity calibration using a white-light source can present experimental difficulties, especially for routine analytical work. Calibrated tungsten halogen lamps have a limited lifetime and require periodic recalibration. These lamps are often mounted in an integrating sphere to eliminate polarization effects and provide uniform source irradiance. In practice, these sources can be difficult to align with the variety of sampling arrangements that are now typical with Raman spectrometers, especially microscope based systems and process Raman analyzers where electrical safety concerns persist in hazardous areas. The advantage of a standard lamp is that it can be used for multiple excitation wavelengths.

4.3 The spectra of materials that luminesce with irradiation can be corrected for relative luminescence intensity as a function of emission wavelength using a calibrated Raman spectrometer. An irradiance source, traceable to the SI, can be used to calibrate the spectrometer. Several groups have proposed these transfer standards to calibrate both Raman and fluorescence spectrometers (**1-6**). The use of a luminescent glass material has the advantage that the Raman excitation laser is used to excite the luminescence emission and this emission is measured in the same position as the sample. These glasses can be used in a variety of sampling configurations and they require no additional instrumentation. The glasses are photostable and unlike primary calibration sources, may not require periodic recalibration. NIST provides a series of fluorescent glasses that may be used to calibrate the intensity axis of Raman spectrometers. A mathematical equation, which is a description of the corrected emission, is provided with each glass. The operator uses this mathematical relation with a measurement of the glass on their spectrometer to produce a system correction curve.

4.4 This guide describes the steps required to produce a relative intensity correction curve for a Raman spectrometer using a calibrated standard source or a NIST SRM and a means to validate the correction.

5. Reagents

5.1 Standard Reference Materials, SRM 2241, SRM 2242, SRM 2243, SRM 2244, SRM 2245, and SRM 2246 are luminescent glass standards designed and calibrated at NIST for the relative intensity correction of Raman spectrometers operating with excitation laser wavelengths of 785 nm, 532 nm, 488 nm/514.5 nm, 1064 nm, 632.8 nm and 830 nm, respectively (**3-5**). The corrected luminescence spectra of each is shown in Fig. 2.

5.2 Raman shift reagents (see Guide E1840).

6. Raman Shift Verification (X-Axis)

6.1 Verification of the calibration of the spectrometer's x-axis in Raman shift wavenumbers ($\Delta \upsilon$ cm^{-1}) is necessary before intensity correction of the y-axis is performed. The Raman shift axis is calculated from Eq 1:

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[5] The boldface numbers in parentheses refer to the list of references at the end of this standard.
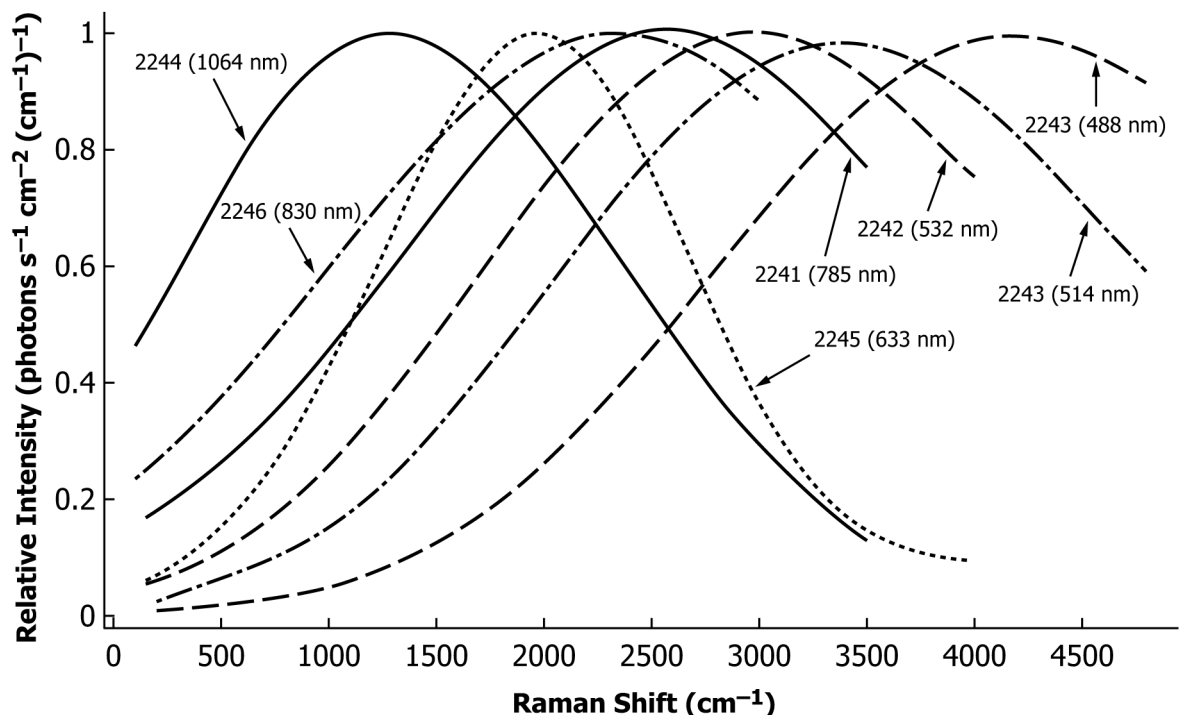
FIG. 2 Certified Models of the Corrected Luminescence Spectra of SRMs 2241 through 2246 as a Function of Raman Shift from the Specified Laser Excitation Wavelength

$$\Delta\upsilon = (\upsilon_0 - \upsilon_s) \tag{1}$$

where:

$\Delta\upsilon$ = the wavenumber in units of Raman shift (cm^{-1}),
υ_0 = the laser frequency in wavenumbers (cm^{-1}),
υ_s = the wavelength axis of the spectrometer expressed in wavenumbers (cm^{-1}),

6.2 The laser frequency can be measured using a wavemeter while the absolute wavenumber axis of the spectrometer is calibrated with emission pen lamps. Several references (**7-10**) have detailed the use of the appropriate emission lamps for the relevant Raman frequency range. Users should defer to the vendor's instructions for the purpose of Raman shift axis calibration or verification. However, independent validation of the Raman shift axis may be performed by referring to Guide E1840.

7. Relative Instrument Response Function Calibration (Y-Axis):

7.1 *General Procedure for Relative Response Calibration:*

7.1.1 The most practical approach to calibrating a relative instrumental response function (IRF) involves the use of a standard of known spectral flux (intensity versus wavelength). The standard source is aligned to the spectrometer such that the emitted optical radiation is directed into the optical path to emulate Raman scattered radiation collected by the spectrometer from the sample position. The best accuracy is achieved when the calibration source radiation and Raman scatter of the sample travel the same illumination path through the collection optics of the spectrometer. The standard source spectrum is measured using, as nearly as possible, the same instrumental parameters (for example, spectral coverage, slit width, filters,

or other optical elements) as used for sample data collection. Excitation laser power, however, is sample dependent and the relative response correction of the spectrometer will be independent of this parameter. Acquisition time should be adjusted to optimize the signal-to-noise ratio (SNR).

7.1.2 The relative IRF is defined as the ratio of the measured spectrum of the standard source, $S_L(\Delta\upsilon)$, to the known standard output, $I_L(\Delta\upsilon)$. The inverse of this relation is used to calculate a relative intensity correction curve as in Eq 2:

$$I_{CORR}(\Delta\upsilon) = I_L(\Delta\upsilon)/S_L(\Delta\upsilon) \tag{2}$$

where:

$I_{CORR}(\Delta\upsilon)$ = the relative intensity correction curve,
$I_L(\Delta\upsilon)$ = the known standard output,
$S_L(\Delta\upsilon)$ = the measured spectrum of the standard source (see 7.1.4).

7.1.3 Once determined, this correction curve is used to correct the measured Raman spectrum of a sample, $S_{MEAS}(\Delta\upsilon)$, for the system dependent response according to Eq 3:

$$S_{CORR}(\Delta\upsilon) = I_{CORR}(\Delta\upsilon) \times S_{MEAS}(\Delta\upsilon) \tag{3}$$

where:

$S_{CORR}(\Delta\upsilon)$ = the corrected Raman spectrum of the sample,
$I_{CORR}(\Delta\upsilon)$ = the relative intensity correction curve,
$S_{MEAS}(\Delta\upsilon)$ = the measured Raman spectrum of the sample (see 7.1.4).

7.1.4 Prior to calculating the relative intensity correction curve or corrected sample Raman spectrum, the measured spectra should be corrected by removing contributions to the signal not originating from the sample or calibration source being measured. Background signal can arise from processes

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independent of light incident on the detector, such as detector bias and thermal charge generation. Correction for these processes is often referred to as dark correction or dark subtraction. Corrections for other sources of background interference, such as ambient lighting or luminescence from optical components in a system, can also be performed. These procedures may be combined into a single measurement and automated during the spectral acquisition. Throughout the remainder of this guide, the term "background" will be used generally to refer to spectral background contributions both dependent and independent of light incident on the detector. The suitability of a particular approach to background correction depends on instrumentation as well as application and, therefore, a specific procedure cannot be universally prescribed (see 7.4.4.1). For the calibration source, the measured spectrum is typically corrected by subtraction of a background spectrum recorded by blocking or removing the calibration source or leaving the detector shutter closed and measuring a spectrum using the same acquisition time used to measure the calibration source. The measured sample spectrum is similarly corrected by subtraction of a background spectrum; however, since the spectrum depends on integration time, this will typically be different from the background spectrum used for the calibration source. Furthermore, the procedure for measuring a background spectrum may differ between a calibration source and sample spectrum. Other variables, such as environmental conditions, can also be important (for example, correction at one temperature may not be universal).

7.1.5 Due to polarization biases that can be present in Raman instrumentation, typically due to the diffraction grating and sample orientation dependent components of the Raman tensor, a polarization scrambler is recommended in the Raman light-collection optics, most preferably in a region of collimated light. Raman spectral bands that exhibit various degrees of polarization will not be properly intensity-corrected without the use of a scrambler (see 7.4.1).

7.1.6 Calibration data for light sources are typically provided in energy output versus wavelength. While the SI unit for spectral irradiance has units of $W\ m^{-3}$, numerous other units are in common use with energy in terms of mW or µW, area in terms of cm^2, and spectral bandpass in terms of cm, µm or nm. Modern Raman spectrometer systems generally count photons and the wavelength axis is expressed in Raman shift wavenumbers ($\Delta\upsilon$, cm^{-1}). More appropriate units in this case are in terms of photon flux as a function of wavenumber (cm^{-1}). A generalized relationship between spectral irradiance in energy versus wavelength and photons versus wavenumbers can be expressed as Eq 4:

$$\Phi_p(\upsilon) = C \cdot \lambda^3 \cdot \Phi_e(\lambda) \tag{4}$$

where:
$\Phi_p(\upsilon)$ = a photon flux in terms of absolute wavenumbers (cm^{-1}),
$\Phi_e(\lambda)$ = an energy flux in terms of wavelength,
C = a constant that depends on the energy and wavelength calibration units.

7.1.7 Conventional units used for Raman spectrometers employing photon counting detectors are photons sec^{-1} $(cm^{-1})^{-1}$ on a relative scale. Additional units relating to area and solid angle may also be included. With the excitation laser wavelength known, the photon flux in terms of Raman shift, $\Phi_p(\Delta\upsilon)$, may be calculated for a spectrometer system. This approach is utilized for the corrected luminescence spectra of the NIST SRM series for relative Raman intensity correction.

7.2 *Relative Response Calibration using NIST SRMs:*

7.2.1 SRMs 2241 through 2246 are glass artifact standards that transfer a relative irradiance calibration from a NIST certified spectral irradiance source to a user's spectrometer. Under laser excitation at the specified wavelength the SRM luminescence provides a source of known relative spectral intensity, described by a certified mathematical model, over the spectral range of certification. Shown in Fig. 2 are the certified models of the corrected luminescence spectra of SRMs 2241 through 2246 as a function of Raman shift from the specified laser excitation wavelength. The corrected spectra of these SRMs were determined by the general response calibration procedure described above using a NIST calibrated primary standard source and/or calibrated black-body furnace radiators. With the SRM a relative intensity correction curve is generated using the certified mathematical model obtained from the appropriate SRM certificate (11) and a measured luminescence spectrum of the glass standard on the spectrometer system.

7.2.2 To acquire the luminescence spectrum of the SRM, the surface of the glass should be placed at approximately the same position from which the Raman spectrum of the sample is collected. It is important that the laser excitation be incident only on the frosted surface of the glass. The shape of the spectral luminescence will have some sensitivity to the placement of the glass surface relative to the collection optics of the spectrometer, which is minimized by scattering from the frosted surface (see 7.4.2). Measurement conditions should be set to obtain a spectrum of optimum signal-to-noise ratio. The luminescence spectrum of the glass must be acquired over the same Raman range as that of the sample and with the same data point density and in the same manner (for example, static, stepping or scanning acquisition modes).

7.2.3 The certified relative spectral intensity of the SRM, $I_{SRM}(\Delta\upsilon)$, is calculated using the certified equation listed in the appropriate SRM certificate. $I_{SRM}(\Delta\upsilon)$ has been normalized to unity and is a relative unit expressed in terms of photons sec^{-1} cm^{-2} $(cm^{-1})^{-1}$. The elements of $I_{SRM}(\Delta\upsilon)$ are obtained by evaluating the certified equation at the same data point spacing used for the acquisition of the luminescence spectrum of the SRM and of the Raman spectrum of the sample. Together with the measured luminescence spectrum of the SRM, $S_{SRM}(\Delta\upsilon)$, the relative intensity correction curve is calculated using Eq 5:

$$I_{CORR}(\Delta\upsilon) = I_{SRM}(\Delta\upsilon)/S_{SRM}(\Delta\upsilon) \tag{5}$$

where:
$I_{CORR}(\Delta\upsilon)$ = the relative intensity correction curve,
$I_{SRM}(\Delta\upsilon)$ = the calculated certified relative spectral intensity of the SRM,
$S_{SRM}(\Delta\upsilon)$ = the measured luminescence spectrum of the SRM.

7.2.4 This relative intensity correction curve, $I_{CORR}(\Delta \upsilon)$, corresponds to that derived in Eq 2 of the general procedure described in 7.1 and the intensity corrected Raman spectrum is calculated as in Eq 3. An example of the procedure for correction of instrumental response variation is illustrated in Fig. 3. Shown in the top two panels on the left are the certified (corrected) spectrum of SRM 2241, $I_{SRM}(\Delta \upsilon)$, generated from the certified model (a 5th degree polynomial for SRM 2241) provided in the SRM certificate and the measured luminescence spectrum of SRM 2241, $S_{SRM}(\Delta \upsilon)$, obtained on a commercial Raman system using 785 nm laser excitation. The system-dependent relative intensity correction curve, $I_{CORR}(\Delta \upsilon)$, calculated using Eq 5, is shown in the middle panel. The bottom two panels show the measured sample Raman spectrum, $S_{MEAS}(\Delta \upsilon)$, and the corrected sample Raman spectrum, $S_{CORR}(\Delta \upsilon)$, calculated as the product $I_{CORR}(\Delta \upsilon) \times S_{MEAS}(\Delta \upsilon)$. Both the SRM and sample spectra were corrected by subtraction of background spectra collected using identical acquisition parameters but with the SRM/sample removed. Comparisons of the uncorrected spectra and corresponding background spectra are shown on the right hand side of Fig. 3. Some minor but discernible structure is observed in the background spectra, which is attributable to luminescence from the optical elements in the excitation and collection optic paths since the excitation laser was on during acquisition of background spectra. In addition, there are contributions from imperfect filtering of Rayleigh scatter.

7.2.5 Fig. 4 shows uncorrected spectra of cyclohexane acquired with four different laser excitation wavelengths. Comparison of the relative intensities of the 801 cm^{-1} band to the –CH stretch region (2800 cm^{-1} to 3000 cm^{-1}) clearly demonstrates the effect of the wavelength dependent instrument response function unique to each spectrometer and excitation wavelength. Shown in Fig. 5 are the Raman spectra in Fig. 4 corrected using the appropriate SRM. For each, the uncorrected sample Raman spectrum, $S_{MEAS}(\Delta \upsilon)$, was multiplied by the system-dependent relative intensity correction curve, $I_{CORR}(\Delta \upsilon)$, to produce the corrected spectrum, $S_{CORR}(\Delta \upsilon)$. In addition, to facilitate comparison of spectra across laser excitation wavelengths, a frequency dependent scatter correction (see 7.3) was applied to each spectrum to reduce them to wavelength independent cross-sections.

7.3 *Validation of the Relative Intensity Correction:*

7.3.1 Once the relative intensity correction curve for a spectrometer system has been obtained, the determination of the ratios of chosen band areas of a measured Raman spectrum has been suggested as a simple means to provide validation of the intensity correction. Cyclohexane is one of the materials selected as a Raman shift standard in Guide E1840, since it has a number of bands that span the Raman shift regions of interest. These same features make cyclohexane a material of interest for intensity validation, and several labs have published the peak area ratios for cyclohexane as well as for a number of these Raman shift standards (6, 7). For validation of the intensity correction using cyclohexane it has been customary to select the five prominent bands and ratio the peak area of these to the area of the 801 cm^{-1} ring breathing mode of cyclohexane. Approximate values of these four bands ratios to

the 801 cm^{-1} band are listed in Table 1 as a function of wavelength of excitation. The integration range for each of the bands is listed in column 2. The first column represents a reference Raman shift value for each band. Listed in column 3 are average ratios obtained from frequency independent cross sections (for example, corrected for the $\upsilon_0(\upsilon_0-\upsilon_i)^3$ scattering dependence) of corrected data using excitation wavelengths 514.5 nm, 532 nm, 632.8 nm and 785 nm. The remaining columns list the theoretical value of the ratio incorporating the $\upsilon_0(\upsilon_0-\upsilon_i)^3$ scattering dependence (7) for various common excitation lasers wavelengths. These laser wavelength dependent peak area ratios relative to the 801-band (A_i/A_{801}) are calculated from the values in column 2 according to Eq 6:

$$\frac{A_i}{A_{801}} = \frac{\sigma_i^o}{\sigma_{801}^o} \frac{(\upsilon_0 - \upsilon_i)^3}{(\upsilon_0 - 801)^3} \tag{6}$$

where:

σ_i^o = the laser independent Raman cross section of band i,
σ_{801}^o = the laser independent Raman cross section of the 801-band,
υ_0 = the excitation laser wavenumber (cm^{-1}, absolute),
υ_i = the position of band i in Raman shift wavenumbers (cm^{-1}).

7.3.2 After correction of the spectrum of cyclohexane with the appropriate SRM, this table can provide a validation of the correction process. Note variance of the integrated band area ratios, especially of the –CH region (2567 to 3068 cm^{-1}) to 801 cm^{-1} region of 20 % or more can be expected. This variance can be due to systematic errors that are uncorrected by the SRM. These include the lack of adequate baseline compensation, polarization dependence of the spectrometer when a depolarizer is not used, and integration errors due to low data point density.

7.4 *Common Issues Encountered Correcting Raman Spectra:*

7.4.1 *Polarization Biases*—For a spectrometer system, a polarization bias is the variation in instrument response to light of different polarization orientations, usually measured at orthogonal polarizations with respect to a reference axis of the instrument. The predominant source of polarization bias in the instrument response is the polarization dependence of the efficiency curves of the diffraction grating. The polarization dependence of diffraction gratings is typically characterized as absolute or relative diffracted efficiency as a function of wavelength for P-plane and S-plane polarizations. P-plane (or TE) polarization is light polarized parallel to the grating grooves and S-plane (or TM) polarization is light polarized perpendicular to the grating grooves. Grating efficiency curves can be rather complex and a detailed discussion is beyond the scope of this document (12). In general, higher dispersion diffraction gratings have greater disparities between the S and P polarization dependent efficiency curves than lower dispersion gratings. Other optical elements, such as mirrors and filters, can also influence the polarization state of reflected or transmitted light. Accordingly, nearly all optical instrumentation exhibits some degree of polarization bias. As a general rule, the polarization orientation of the excitation light is maintained to some extent in Raman scattered light (the
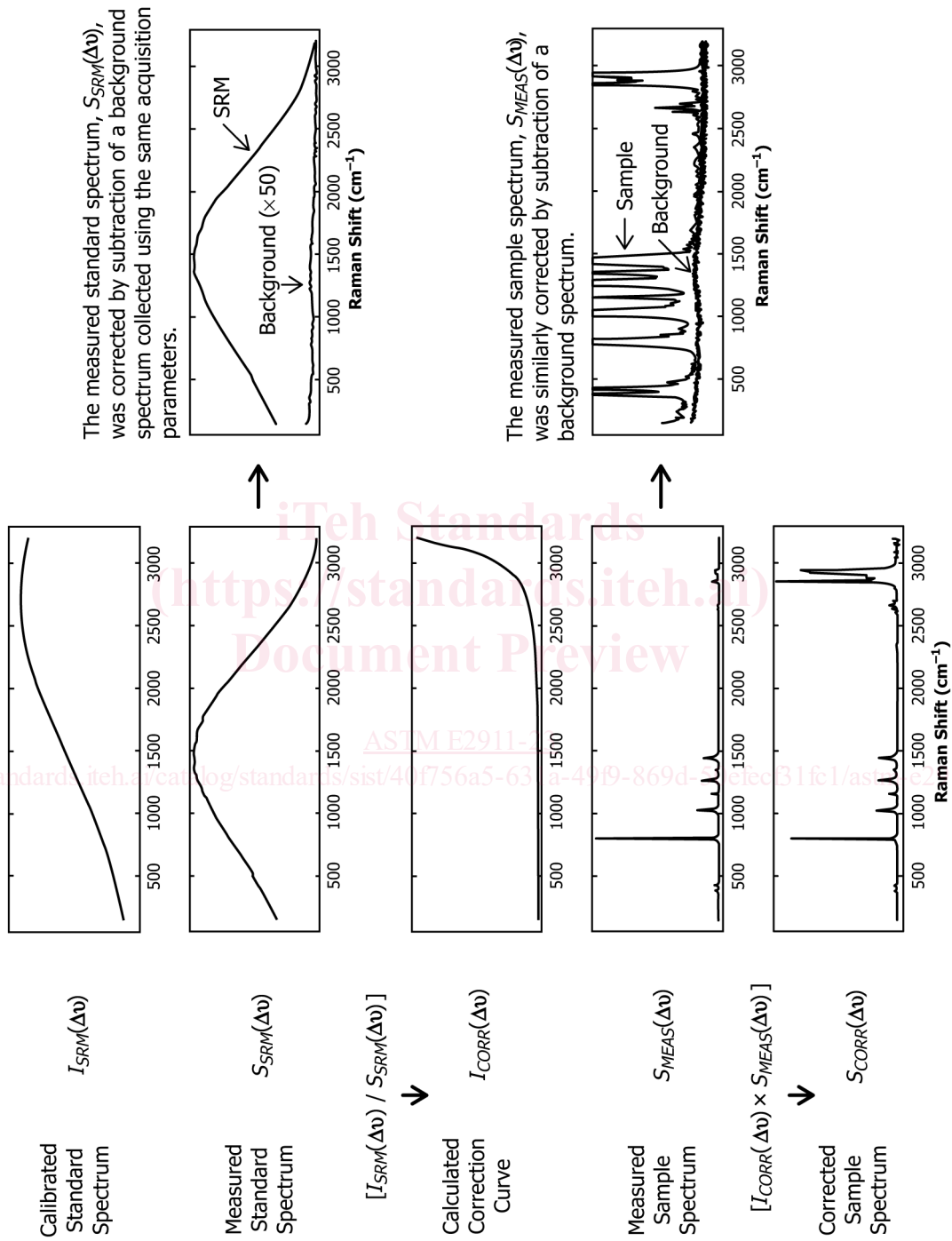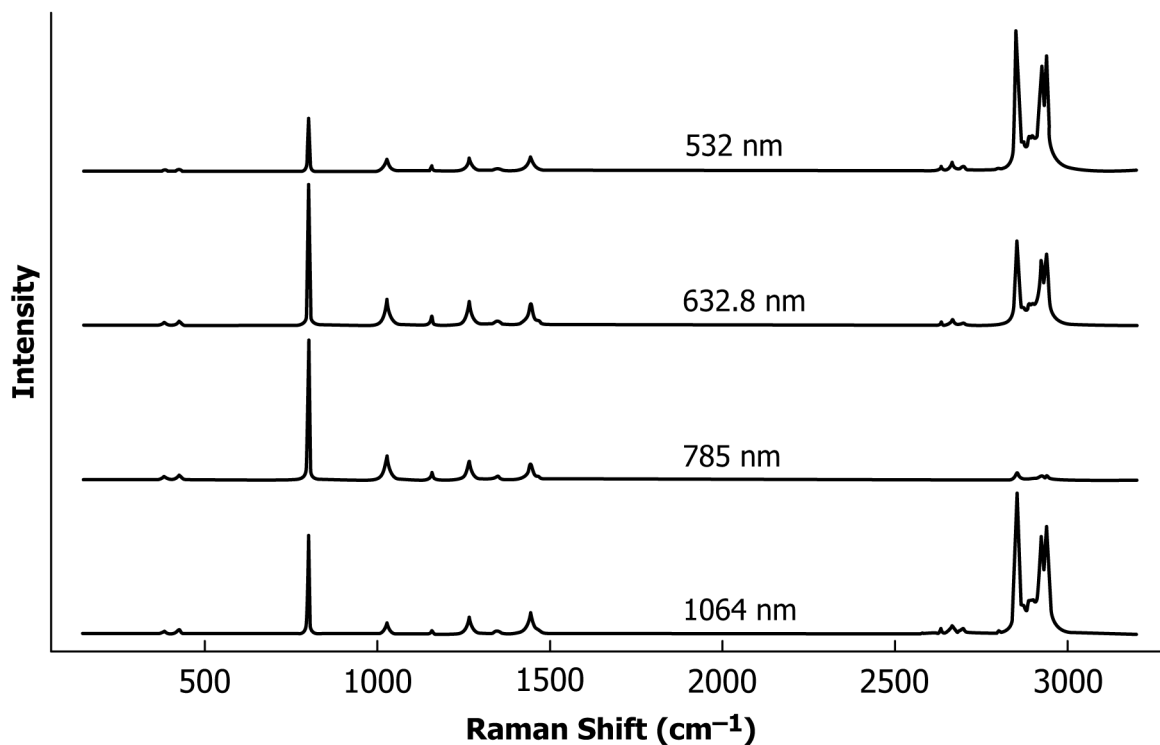
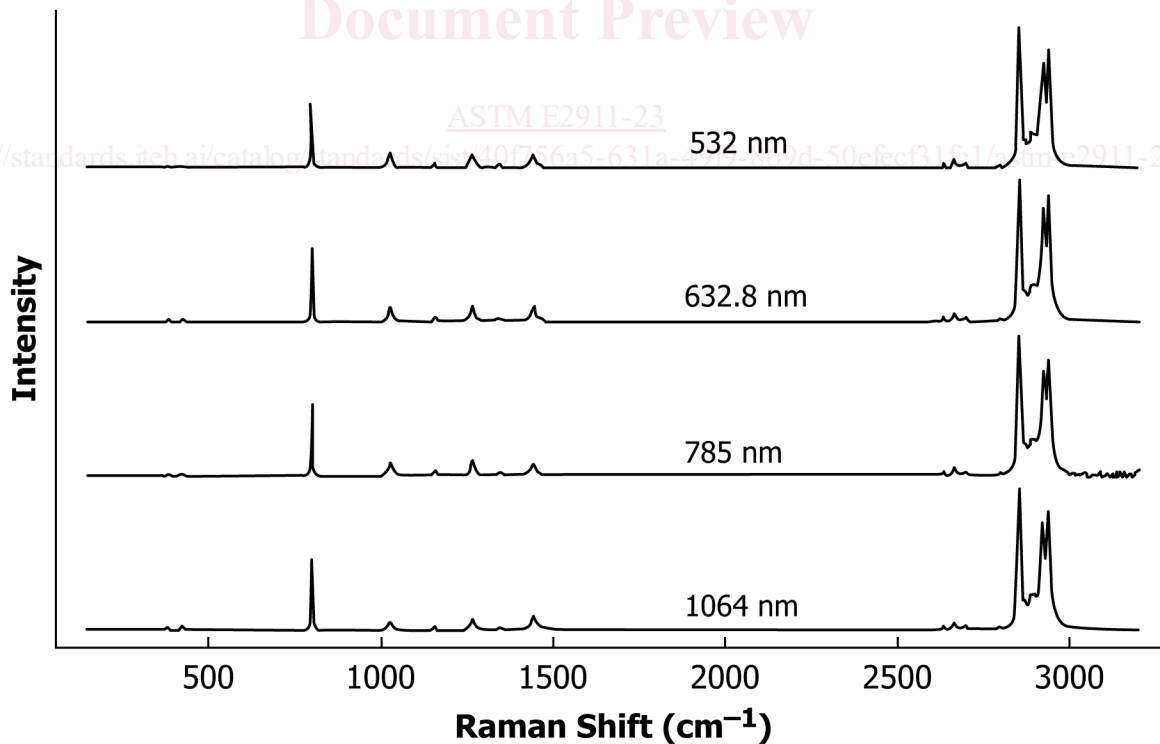FIG. 3 Example of the Procedure for the Correction of Instrumental Response

The spectra have been normalized to maximum of unity and offset for comparison.

FIG. 4 Uncorrected Raman Spectra of Cyclohexane Measured with Four Different Excitation Laser Wavelengths:
532 nm, 632.8 nm, 785 nm, 1064 nm

The spectra have been normalized to maximum of unity and offset for comparison.

FIG. 5 Corrected Raman Spectra of Cyclohexane Using SRM 2242 (532 nm), SRM 2245 (632.8 nm), SRM 2241 (785 nm),
and SRM 2244 (1064 nm)